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Use of Antigens Derived from *Burkholderia pseudomallei*, *B. thailandensis*, and *B. cepacia* in the Indirect Hemagglutination Assay for Melioidosis[▽]

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The serological diagnosis of melioidosis is carried out using the indirect hemagglutination assay. We looked at the reactivity of sera from culture-proven cases of melioidosis from north Queensland against antigens derived from *Burkholderia pseudomallei*, *B. thailandensis*, and *B. cepacia*. Cross-reactivity between sera from culture-positive cases of melioidosis and *B. thailandensis* was demonstrated.

Melioidosis is an important cause of morbidity and mortality in northern Australia and Southeast Asia. Diagnosis is best made by isolating the causative agent, *Burkholderia pseudomallei*, from clinical specimens (6, 7, 9). A variety of clinical presentations are described here, with pneumonia being the most common. Other presentations are diverse and include prostatic abscesses, hepatosplenic abscesses, septic arthritis, osteomyelitis, neurological disease, parotitis, and skin involvement. Acute melioidosis commonly presents as a fulminant septicemia, often resulting in death within a few days of exposure (6).

Serology has a particular role in subacute presentations and in the screening of patients with a febrile illness who have returned from an area of endemicity. The indirect hemagglutination assay (IHA) is the most common serological test used, with titers of 1:40 or greater being considered reactive (1, 2, 3). The IHA is considered both sensitive (between 50 and 80%) and specific (92%) (4). High rates of background seropositivity in some communities would reduce the sensitivity of the assay. Background seropositivity in north Queensland has been reported to be between 5 to 7% (3). In northeastern Thailand, seropositivity in children more than 4 years of age approaches 80% (8).

The antigen used in the IHA is a polysaccharide component of the slime layer of the organism. A variety of polysaccharide and lipopolysaccharide antigens have been used in the IHA. A recent study looked at the higher background seropositivity in Thailand compared with that in Australia (10). The authors postulated that this result was due to greater exposure to *B. thailandensis* in Thailand, resulting in higher cross-reactive antibodies. They looked at comparative IHA titers in febrile subjects from Thailand by using *B. pseudomallei*, *B. thailandensis*, and *B. mallei* polysaccharide antigens. Although there was, as expected, considerable cross-reactivity with sera with *B. mallei* and *B. pseudomallei* antigen preparations, surprisingly, there was no significant cross-reactivity with sera between *B. thailandensis* and *B. pseudomallei* antigen preparations in the Thai population.

B. thailandensis has not been isolated in Australia. It would be expected, therefore, that antibodies to this organism would also not be detected in this population. The detection of antibodies to *B. thailandensis* in a healthy population from an area of endemicity in Australia could suggest that the organism was present in the Australian environment. Alternatively, the detection of antibodies to *B. thailandensis* in only patients with culture-proven melioidosis would suggest that there are either antigenic differences or differences in antibody responses between Australian and Thai isolates or patients, respectively.

The aim of this study was to compare IHA titers obtained using a panel of antigens derived from *B. pseudomallei* NCTC 13178 (Australian isolate), *B. thailandensis*, clinical isolates of *B. pseudomallei* from Papua New Guinea and Sri Lanka, and *B. cepacia* with IHA titers obtained using an in-house pool of five *B. pseudomallei* antigens. A panel of sera from both culture-positive and healthy individuals living in a region of endemicity in north Queensland was used. The following organisms were recovered from storage at −70°C, subcultured on Columbia horse blood agar, incubated at 37°C for 24 h, and checked for purity: *B. pseudomallei* NCTC 13178, *B. pseudomallei* clinical isolates from Papua New Guinea (145; QHPS Townsville collection) and Sri Lanka (148; QHPS Townsville collection), *B. thailandensis* (E111; Western Australian Culture Collection), and *B. cepacia* (clinical isolate; QHPS Townsville). The identity of all clinical isolates was confirmed by API 20 NE (bioMerieux).

The IHA was performed as described previously by using sheep erythrocytes (1). For antigen preparation, single colonies of the organisms were inoculated into 150 ml of protein-free medium and incubated on a shaker at 35°C for 2 weeks. The cultures were then autoclaved at 121°C for 15 min. The broths were then centrifuged, and the antigen-containing supernatant was filtered using Millipore 0.45-μm-pore-size filters. Phenol was added to the filtered supernatant to a concentration of 0.5% by volume. Each antigen was then titrated in an IHA with known positive sera to determine the lowest

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TABLE 1. Concordance between the test antigens and the pooled in-house antigens using culture-positive sera[a]

Isolate from which the antigen was derived (country of origin)	No. (%) of serum samples for which the titer was:				No. (%) of results that were[d]:		r (CI)[e]	P[e]
	Same[b]	±1 dilution[c]	±2 dilutions[c]	±3 or more dilutions[c]	Concordant	Discordant		
B. pseudomallei NCTC 13178 (Australia)	9 (36)	10 (40)	3 (12)	1 (4)	23 (92)	2 (8)	0.8135 (0.6088–0.9167)	<0.0001
B. pseudomallei 145 (Papua New Guinea)	9 (36)	8 (32)	5 (20)	1 (4)	23 (92)	2 (8)	0.8506 (0.6795–0.9340)	<0.0001
B. pseudomallei 148 (Sri Lanka)	8 (32)	11 (44)	1 (4)	4 (16)	24 (96)	1 (4)	0.7690 (0.5281–0.8953)	<0.0001
B. thailandensis	5 (20)	4 (16)	5 (20)	8 (32)	22 (88)	3 (12)	0.1802 (−0.2432–0.5469)	<0.3887

[a] The number of serum samples was 25. The range of titers was from 1:10 to more than 1:5,120. CI, confidence interval.
[b] Concordance with the in-house IHA implies that an identical IHA titer was obtained using the antigen named.
[c] Concordance within ±1, ±2, or ±3 (or more) dilutions implies that the IHA titer obtained using the antigen named was either above or below the titer obtained in the in-house IHA by that factor.
[d] A discordant result implies that the IHA result obtained using the antigen named was negative, while the in-house test result was positive. Sera tested using the *B. cepacia* antigen were uniformly negative.
[e] For concordant results, Spearman rank correlation was carried out to obtain r and P values.

concentration of antigen that would give a reproducible titer. This titer was then used for that particular antigen for comparison with the in-house IHA. The antigen used in the in-house IHA was derived from five pooled Australian isolates of *B. pseudomallei*.

A total of 48 serum samples were tested. These represented 25 serum samples from culture-positive patients who had in-house IHA titers ranging from 1:10 to more than 1:5,120. The remaining 23 serum samples were from individuals negative by IHA who had no history of melioidosis. All sera were from residents of north Queensland.

Optimal antigen dilutions were similar for all *B. pseudomallei* antigens, and *B. pseudomallei* was used at a dilution of 1:320. *B. thailandensis* was used at an antigen dilution of 1:10.

There was no reactivity against *B. cepacia* antigen with either culture-positive sera or negative control sera. Control sera negative by IHA also had negative results with all four other antigens tested. Details of the concordance of IHA titers obtained with culture-positive sera by using the four antigens tested are given in Table 1.

High background rates of seropositivity in regions of endemicity have limited the usefulness of the IHA in the diagnosis of melioidosis. In north Queensland, an average of 5.7% of 9,047 random individuals from North Queensland had a titer of 1:40 or greater for the IHA (3).

The first report stating that *B. thailandensis* was a species distinct from *B. pseudomallei* appeared in 1998 (5). Prior to this report, *B. thailandensis* was considered to be a nonpathogenic variant that assimilated the sugar L-arabinose; this is in contrast to the normally pathogenic *B. pseudomallei*, which does not. *B. thailandensis* is present in the environment in Thailand, where clinical melioidosis is endemic. Conversely, in northern Australia, where melioidosis is also endemic, *B. thailandensis* has yet to be isolated from the environment. The presence of *B. thailandensis* in the environment has been suggested as a reason for the higher background seropositivity seen in Thailand. However, a recent study from Thailand demonstrated that antibodies to *B. thailandensis* were not detected in sera from 84% of culture-confirmed cases of melioidosis (10). The explanation given for this apparent discrepancy was either that environmental exposure to *B. thailandensis* did not result in the generation of antibodies or that antibodies were not recognized by the antigens in the IHA.

Our findings differ from those of the study mentioned above in that reactivity to *B. thailandensis*, while not statistically significant ($r = 0.1802$ [Spearman correlation], $P = 0.3887$), was seen in 88% of culture-positive sera. In addition, there was no cross-reactivity shown with *B. cepacia*.

Two significant points in our study should, however, be noted. First, reactivity to *B. thailandensis* was seen only at a lower antigen dilution (1:10 dilution) than that used with the other *B. pseudomallei* antigens (1:320 dilution). Second, concordance within 1 dilution was seen in only 36% of sera tested with *B. thailandensis* antigen, while it was seen in up to 78% of sera tested with *B. pseudomallei* antigen. The absence of any reactivity with the control sera would suggest that this concordance was not due to nonspecific cross-reactivity. We acknowledge that a larger cohort of control sera needs to be tested to further confirm this observation. In this study, the geographic source of the *B. pseudomallei* used as antigen was also shown to have no effect on the IHA titer. Traditionally, it has been suggested that the IHA needs to be performed by using pooled antigens from a variety of sources. This study demonstrates that there is little difference in IHA titers obtained using geographically diverse isolates of *B. pseudomallei.*

This study demonstrates that sera from culture-positive patients with melioidosis in North Queensland will cross-react with *B. thailandensis* used at a different IHA antigen concentration. This result may indicate intrinsic differences in specific antigens between the two species and warrants further investigation.

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